

TM 1326 – AK AGAR NO. 2 (SPORULATING AGAR) (ARRET AND KIRSHBAUM MEDIUM)

INTENDED USE

For production of spores of *Bacillus subtilis* ATCC 6633 which are used as inoculum in detection of Penicillin and other antibiotics.

PRODUCT SUMMARY AND EXPLANATION

Detection of penicillin and other antibiotic residues in milk is of primary importance in the dairy industry. This is done by the Penicillin Milk Test procedure. AK Agar formulated by Arret and Kirshbaum is used for the production of spores of *Bacillus subtilis* ATCC 6633, which is used in the Penicillin Milk Test procedure.

A fresh slant culture of *Bacillus subtilis* is washed with sterile physiological saline onto the surface of Roux bottles containing 300 ml sterile medium. The bottles are incubated at 35°C for 5 days and the resulting growth is suspended into 50 ml of sterile physiological saline. The growth is washed by centrifuging the suspension and discarding the supernatant. The sediment obtained is re-suspended in fresh sterile saline and heated at 70°C for 30 minutes to kill vegetative cells and obtain the spore suspension. This spore suspension can be stored for months for use in detection of penicillin/ antibiotic residues in milk and dairy products.

COMPOSITION

Ingredients	Gms / Ltr
Gelatin peptone	6.000
Tryptone	4.000
Yeast extract	3.000
Beef extract	1.500
Dextrose (Glucose)	1.000
Manganous sulphate	0.300
Agar	15.000

PRINCIPLE

This medium is highly nutritious due to the presence of yeast extract, beef extract, gelatin peptone and tryptone in addition to being a source of vitamins and essential amino acids. Dextrose serves as the source of energy as well as the fermentable carbohydrate. Manganous sulphate stimulates sporulation.

INSTRUCTION FOR USE

- Dissolve 30.8 grams in 1000 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Dispense in 300 ml amounts in Roux or other suitable bottles.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.

Note: Do not autoclave till the medium has been completely dissolved.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Light amber coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C)	: 6.6±0.2



INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Sporulation	Incubation Temperature	Incubation Period
Bacillus megaterium	25848	50-100	Good-luxuriant	>=50%	Positive	35-37°C	5 Days
Bacillus subtilis subsp. spizizenii	6633	50-100	Good-luxuriant	>=50%	Positive	35-37°C	5 Days

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.













Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- Arret and Kirshbaum, 1959, J. Milk and Food Tech., 22:329.
- Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
- Richardson (Ed.), 1995, Standard Methods for the Examination of Dairy Products, 15th Ed., APHA, Washington D.C.

 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Birkstrasse 10 48163 Muenster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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